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(54) Title: HUMAN CELLULAR PROTEIN KINASES, METALLOPROTEASES AND PHOSPHATASES AS TARGETS FOR MEDICAL INTERVENTION AGAINST HEPATITIS C VIRUS INFECTIONS

(57) Abstract: The present invention relates to human cellular protein kinases, metalloproteases and one phosphatase: beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649) as potential targets for medical intervention against Hepatitis C virus (HCV) infections. The present invention relates also to a method for the detection of compounds useful for prophylaxis and/or treatment of Hepatitis C virus infections, a method for detecting Hepatitis C virus infections in an individual or in cells. Mono- or polyclonal antibodies are disclosed effective for the treatment of HCV infections together with methods for treating Hepatitis C virus infections or for the regulation of Hepatitis C virus production and/or replication wherein said antibodies may be used. Finally the present invention relates to a solid support useful for detecting Hepatitis C virus infections or for screening compounds useful for prophylaxis and/or treatment of HCV infections.

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Human cellular protein kinases, metalloproteases and phosphatases as targets for medical intervention against Hepatitis C virus infections

Specification

The present invention relates to the human cellular protein kinases, metalloproteases and phosphatases:

1. beta-adrenergic receptor kinase 1 (NM_001619, X61157, ADRBK1, GRK2, BARK1);
2. Mitogen activated protein kinase activated protein kinase 5 (AF032437, MAPKAPK5, PRAK,);
3. Insulin-stimulated protein kinase 1 (U08316, ribosomal protein S6 kinase 3 90K);
4. Discoidin domain receptor family, member 1 (NM_013994, DDR1, TRK E, NEP, CAK, X74979);
5. Protein Kinase C, mu (X75756, PKC-mu);
6. Protein Kinase C, theta (L01087, PKC-theta);
7. AMP-activated protein kinase beta 2 subunit (AJ 224538, AMPK beta 2);
8. JNK2 (U09759, L31951);
9. Human p21-activated protein kinase 2 (U24153, PAK2);
10. cyclin-dependent kinase 4 (U37022, cdk4);
11. MEK5 (U25265, Mitogen-activated protein kinase kinase 5);
12. MKP-L or DUSP14, formally also named as MKP-1 like tyrosine phosphatase (NM_007026, AF038844);
13. ADAM22 (NM_016351, AF155382; a disintegrin and metalloproteinase domain 22);
14. ADAM17 (U92649, XM_002270; a disintegrin and metalloproteinase domain 17);

as potential targets for medical intervention against Hepatitis C virus (HCV) infections. Furthermore, the present invention relates to a method for the detection of compounds useful for prophylaxis and/or treatment of Hepatitis C

virus infections and a method for detecting Hepatitis C virus infections in an individual or in cells. Also mono- or polyclonal antibodies are disclosed effective for the treatment of HCV infections together with methods for treating Hepatitis C virus infections or for the regulation of Hepatitis C virus production wherein said antibodies may be used. Finally the present invention relates to a solid support useful for detecting Hepatitis C virus infections or for screening compounds useful for prophylaxis and/or treatment of HCV infections.

Background of the invention

Hepatitis C Virus (HCV) infection is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma. The WHO estimates that approximately 3% of the world population, or 170 million people, have been infected with the Hepatitis C Virus. In the U.S., an estimated 3.9 million Americans have been infected (CDC fact sheet Sept. 00). Over 80% of HCV-infected individuals develop chronic hepatitis, which is associated with disease states ranging from asymptomatic carrier states to repeated inflammation of the liver and serious chronic liver disease. Over the course of 20 years, more than 20% of chronic HCV-patients are expected to be at risk to develop cirrhosis or progress to hepatocellular carcinoma. Liver failure from chronic hepatitis C is the leading indicator for liver transplantation. Excluding transplantation, the CDC estimates that medical and work-loss cost for HCV annually are around $600 million. HCV is transmitted primarily by blood and blood products. Due to routine screening of the blood supplies from mid-1992, new transfusion-related cases are exceedingly rare and have been surpassed by injection drug use as the highest risk factor for acquiring the virus. There is also a sexual, however inefficient, route of transmission, and a 6% rate of transmission from infected mothers to their children, which is higher in case of HIV coinfection. In a certain percentage of infections, the mode of transmission remains unknown. In spite of the significant decline in incidence in the 1990's, the number of deaths (estimated deaths annually at the moment: 8000 to 10,000 in U.S.) and severe disease due to HCV is anticipated to triple in the next 10 to 20 years. (Sources: CDC fact sheet (accessed 12/12/00); Houghton M. Hepatitis C Viruses. In BN Fields, DM Knipe, PM Howley (ed.) Fields Virology.

1996. Lippencott-Raven Pub., Philadelphia; Rosen HR and Gretch DR, Molecular Medicine Today Vol5, 393, Sept. 99; Science 285, 26, July 99: News Focus: The scientific challenge of Hepatitis C; Wong JB et al, Am J Public Health, 90, 1562, Oct 2000: Estimating future hepatitis C morbidity, mortality, and costs in the United States).

According to the Consensus Statement from the EASL (European Association for the Study of the Liver) International Consensus Conference on Hepatitis C (February 26-28, 1999, Paris, France), combination therapy of alpha interferon and ribavirin is the recommended treatment for naive patients. Monotherapy with interferon has also been approved by the FDA, but the sustained response rate (HCV RNA remains undetectable in the serum for more than 6 months after end of therapy) is only 15 to 20%, in contrast to 35 to 45% with combination therapy. Interferons (Intron A, Schering-Plough; Roferon A, Hoffmann-LaRoche; Wellferon, Glaxo Wellcome; Infergen, Amgen) are injected subcutaneously three times a week, ribavirin (Rebetol, Schering-Plough) is an oral drug given twice a day. Recommended treatment duration is 6 to 12 months, depending on HCV genotype. Experimental forms of slow-release pegylated interferons (Pegasys, Hoffmann-LaRoche; PEG-Intron, Schering-Plough) have shown improvements in response rates (42 to 82% in combination with ribavirin) and application (once-weekly injection) in recent clinical studies (Hepatology32:4, Pt 2 of 2. Oct 2000; NEJM 343, 1673. Dec 00; NEJM 343, 1666. Dec 00). Common side effects of interferon therapy include: fatigue, muscle aches, head aches, nausea, fever, weight loss, irritability, depression, bone marrow suppression, reversible hair loss. The most common side effects of ribavirin are anemia, fatigue and irritability, itching, skin rash, nasal stuffiness, sinusitis, cough. More serious side effects of mono-and combination therapy occur in less than two percent of patients (NIDDK information: Chronic Hepatitis C: Current Disease Management. accessed 09.12.99). Some of the contraindications to interferon are psychosis or severe depression; neutropenia and/or thrombocytopenia; organ transplantation except liver; symptomatic heart disease; decompensated cirrhosis; uncontrolled seizures. Contraindications to ribavirin are end-stage renal failure; anemia;

hemoglobinopathies; severe heart disease; pregnancy; no reliable method of contraception (consensus statement EASL).

Experimental treatments that are not new forms of interferon are Maxamine (histamine dihydrochloride, Maxim Pharmaceuticals), which will be combined with Interferon in phase III studies, VX-497 (Vertex Pharmaceuticals), an IMP dehydrogenase inhibitor, as a less toxic ribavirin substitute in phase II, and amantadine (Endo Labs), an approved influenza drug, as the third component in triple therapy (phase II). Inhibitors for HCV enzymes such as protease inhibitors, RNA polymerase inhibitors, helicase inhibitors as well as ribozymes and antisense RNAs are under preclinical development (Boehringer Ingelheim, Ribozyme Pharmaceuticals, Vertex Pharmaceuticals, Schering-Plough, Hoffmann-LaRoche, Immusol, Merck etc.). No vaccine is available for prevention or therapeutic use, but several companies are trying to develop conventional or DNA vaccines or immunostimulatory agents (e.g. Chiron, Merck/Vical, Epimmune, NABI, Innogenetics).

In summary, the available treatment for chronic Hepatitis C is expensive, effective only in a certain percentage of patients and adverse side effects are not uncommon.

Description of the invention

Recent research has revealed how cells communicate with each other to coordinate the growth and maintenance of the multitude of tissues within the human body. A key element of this communication network is the transmission of a signal from the exterior of a cell to its nucleus, which results in the activation or suppression of specific genes. This process is called signal transduction.

An integral part of signal transduction is the interaction of ligands, their receptors and intracellular signal transduction molecules. Ligands are messengers that bind to specific receptors on the surface of target cells. As a result of the binding, the receptors trigger the activation of a cascade of downstream signaling molecules,

thereby transmitting the message from the exterior of the cell to its nucleus. When the message reaches the nucleus, it initiates the modulation of specific genes, resulting in the production of RNA and finally proteins that carry out a specific biological function. Disturbed activity of signal transduction molecules may lead to the malfunctioning of cells and disease processes. Specifically, interaction of HCV with host cells is necessary for the virus to replicate.

The present invention is based upon the discovery of a group of human cellular protein kinases, metalloproteases and one phosphatase which are specifically up- or downregulated as a result of HCV replication in HCV infected host cells. The antiviral therapeutic research approach described herein focuses on discovering the cellular signal transduction pathways involved in viral infections. Identification of the signal transduction molecules, key to viral infection, provides for, among other things, novel diagnostic methods, for example, assays and compositions useful therefore, novel targets for antiviral therapeutics, a novel class of antiviral therapeutics, and new screening methods (e.g. assays) and materials to discover new antiviral agents.

This approach led to the development of a novel microarray platform technology, wherein a microarray of more than 900 signal transduction cDNAs was developed. This unique microarray technology was used to identify changes in RNA expression patterns (e.g. upregulation or downregulation) as a result of HCV infected host cells. Differential display techniques were used to pinpoint those signal transduction molecules useful as targets for drug intervention. Effective manipulation of these virally-controlled intracellular signal transduction pathways can alter (slow or stop altogether) the course of viral growth.

It is object of the present invention to provide novel targets for medical intervention, prophylaxis and/or treatment of Hepatitis C virus infections in mammals, including humans, and cells together with methods for detecting HCV infections in individuals and cells and methods for detecting compounds useful for prophylaxis and/or treatment of HCV infections. The object of the present

invention is solved by the teaching of the independent claims. Further preferred embodiments are disclosed in the dependent claims.

It is now revealed for the first time that the human cellular proteins beta-adrenergic receptor kinase 1 (gene accession number NM_001619, other names: X61157, ADRBK1, GRK2, BARK1),
Mitogen activated protein kinase activated protein kinase 5 (also known as: AF032437, MAPKAPK5, or PRAK),
Insulin-stimulated protein kinase 1 (also known as: U08316, ribosomal protein S6 kinase 3 90K),
Discoidin domain receptor family, member 1 (gene accession number NM_013994, other names: X74979, DDR1, TRK E, NEP, or CAK),
Protein Kinase C, mu (also known as: X75756 or PKC-mu),
Protein Kinase C, theta (also known as: L01087 or PKC-theta),
AMP-activated protein kinase beta 2 subunit (also known as: AJ 224538 or AMPK beta 2),
JNK2 (also known as: U09759 or L31951),
Human p21-activated protein kinase 2 (also known as: U24153 or PAK2),
cyclin-dependent kinase 4 (also known as: U37022 or cdk4),
MEK5 (also known as: U25265 or Mitogen-activated protein kinase kinase 5),
MKP-L (gene accession number: NM_007026; also known as AF038844),
ADAM22 (gene accession number: NM_016351, a disintegrin and metalloproteinase domain 22, also known as AF155382),
ADAM17 (also known as: U92649 or a disintegrin and metalloproteinase domain 17 (tumor necrosis factor, alpha, converting enzyme) also known as XM_002270), are specifically and uniquely up- or downregulated in a cell as a result of HCV infection. These cellular protein kinases, metalloproteases and the phosphatase therefore identify novel diagnostic and therapeutic targets for HCV infection.

The only reliable experimental HCV infection studies have been performed with chimpanzees. There is no simple cell culture infection system available for HCV. Although a number of reports have been published describing *in vitro* propagation attempts of HCV in primary cells and cell lines, questions remain concerning

reproducibility, low levels of expression and properly controlled detection methods (reviewed in J. Gen Virol. 81, 1631; Antiviral Chemistry and Chemotherapy 10, 99). Thus, the replicon system described by Bartenschlager and coworkers (Lohmann et al, Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 285, 110. 1999) was used for the studies disclosed herein. This replicon system reproduces a crucial part of the HCV replication cycle which is used as a system for simulating HCV infection. Bartenschlager's group produced bicistronic recombinant RNAs, so-called "replicons", which carry the Neomycin-phosphotransferase gene as well as a version of the HCV genome where the sequences for the structural HCV proteins were deleted. After transfection of the subgenomic HCV RNA molecules into the human hepatoma cell line Huh-7, cells supporting efficient RNA-dependent RNA replication of the HCV replicons were selected based on co-amplification of the neo gene and resulting resistance to the antibiotic G418. Integration of coding information into the cellular genome was an exclusion criterium for functional replicons. Several lines were established from G418 resistant clones with autonomously replicating HCV RNAs detectable by Northern blot. Minus-strand RNA replication intermediates were detected by Northern blot or metabolic radio-labeling, and the production of nonstructural HCV proteins was demonstrated by immuno-precipitation after metabolic labeling or Western blot.

Possible influences and/or dependencies of HCV's RNA-dependent RNA replication and nonstructural proteins on host cell transcription are accessible to analysis with the cDNA arrays used in the inventive methods described herein. Expression levels can be confirmed using Northern or Taqman analysis at the RNA and Western blot analysis at the protein level. Huh-pcDNA3 cells are Huh7 cells resistant to G418 by integration of a plasmid and serve as negative control. Three replicon lines were analyzed for changes in cellular RNA expression patterns compared to the control line:

- Huh-9-13: cell line with persistant replicon I377/NS3-3'/wt, described in Science 1999, 285, 110-113,
- Huh-5-15: cell line with persistant replicon I389/NS3-3'/wt, described in Science 1999, 285, 110-113,
- Huh-11-7: cell line with persistant replicon I377/NS2-3'/wt, described in Science 1999, 285, 110-113.

Based on the discoveries reported herein, one aspect of the present invention is directed to a screening method for detecting compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infections. Specifically, this method involves contacting a test compound with at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta(L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (AF038844, NM_007026), ADAM22 (NM_016351), ADAM17 (U92649) and detecting the human cellular protein kinase, metalloprotease or phosphatase activity.

Another aspect of the present invention is directed to a diagnostic method, an assay for detecting Hepatitis C virus infections in an individual or cells. This method involves providing a sample from the individual or providing cells and detecting activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of: beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), ADAM17 (U92649) and detecting the human cellular protein kinase, metalloprotease or phosphatase activity.

Accordingly, one aspect of the present invention is directed to novel compounds useful in the above-identified methods. Therefore, the present invention relates to a monoclonal or polyclonal antibody that binds to a human cellular protein kinase,

metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351) and ADAM17 (U92649).

Furthermore, the present invention discloses a method for treating Hepatitis C virus infection in an individual comprising the step of administering a pharmaceutically effective amount of an agent which inhibits at least partially the activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

Another object of the present invention is to provide a method for regulating the production of Hepatitis C virus in cells comprising the step of administering a pharmaceutically effective amount of an agent to said cells wherein said agent inhibits at least partially the activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L

(NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649). The above-mentioned monoclonal or polyclonal antibodies directed against these targets may be used as pharmaceutically active agents within said methods.

In order to identify HCV infections and new inhibitors and new pharmaceutically active compounds against Hepatitis C viruses a further aspect of the present invention is directed to a solid support useful for detecting Hepatitis C virus infections in an individual or in cells comprising an immobilized oligonucleotide, wherein said oligonucleotide is capable of detecting activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649). Said solid support is also useful to screen compounds for the prophylaxis and/or treatment of Hepatitis C virus infections in an individual comprising at least one immobilized oligonucleotide, wherein said oligonucleotide encodes one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649) or comprising at least one immobilized human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein

Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

Yet another aspect of the present invention is directed to a novel therapeutic composition useful for the prophylaxis and/or treatment of an individual afflicted with Hepatitis C virus comprising at least one agent capable of inhibiting activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

Detailed description of the invention

Utilizing microarray technology, a unique microarray of more than 900 signal transduction cDNAs was developed. This array was used to compare signal transduction mRNA expression patterns (e.g. upregulation or downregulation) from HCV Replicon cells Huh-9-13, Huh-5-15, and Huh-11-7 with Huh-pcDNA control cells which do not contain HCV Replicons. These HCV Replicon cells serve as a system for simulation of HCV infected cell systems, especially for simulating HCV infected mammals, including humans. Interference of HCV with the cellular signaling events is reflected in differential gene expression when compared to cellular signaling in control cells. Results from this novel signal transduction microarray analysis revealed significant up- or downregulation of human cellular protein kinases, metalloproteases and one phosphatase. Radioactively labeled complex cDNA-probes from HCV Replicon cells Huh-9-13, Huh-5-15, and Huh-11-7 were hybridized to cDNA-arrays and compared to

hybridizations with cDNA-probes from Huh-pcDNA control cells which did not contain HCV Replicons. Surprisingly it was found that the following cellular targets are significantly up- or downregulated:

beta-adrenergic receptor kinase 1 (NM_001619):	2.7 – 3.5 fold stronger
Mitogen activated protein kinase activated protein kinase 5 (AF032437):	2.2 –3.0 fold stronger
Insulin-stimulated protein kinase 1 (U08316):	2.2 – 3.1 fold stronger
TRK E (NM_013994):	3.2 -10.3 fold stronger
Human p21-activated protein kinase 2 (U24153):	1.8 –2.7 fold stronger
PKC-mu (X75756):	2.3 –3.2 fold weaker
PKC-theta (L01087):	2.6 –3.3 fold weaker
AMP-activated protein kinase beta 2 subunit (AJ 224538):	1.9 – 2.2 fold weaker
JNK2 (U09759):	2.6 –4.1 fold weaker
cdk4 (U37022)	1.8 – 3.3 fold stronger
MEK5 (U25265)	0.9 –3.6 fold stronger
MKP-L (NM_007026):	2.1 –2.3 fold weaker
ADAM22 (NM_016351):	2.5 –3.8 fold weaker
ADAM17 (U92649):	3.4 –3.8 fold weaker

Disclosed herein is the first report describing the role of human cellular proteins beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein

kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), ADAM17 (U92649) in the signal transduction of the HCV infection process. As a result of these discoveries, novel compounds and inhibitors against the above-mentioned human cellular protein kinases, metalloproteases and phosphatases may be found by the use of the inventive methods disclosed herein.

ADAM17 and ADAM22 are proteins of the ADAM family (proteins containing a disintegrin and metalloprotease domain). ADAM17 is also known as "Homo sapiens a disintegrin and metalloproteinase domain 17" and ADAM22 is know as "Homo sapiens metalloprotease-like, disintegrin-like, cysteine-rich protein 2 epsilon".

As used herein, the term "inhibitor" refers to any compound capable of downregulating, decreasing, suppressing or otherwise regulating the amount and/or activity of the human cellular proteins beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649). Generally, human cellular protein inhibitors may be proteins, oligo- and polypeptides, nucleic acids, small chemical molecules, or other chemical moieties.

As used herein, the term "regulating expression and/or activity" generally refers to any process that functions to control or modulate the quantity or activity (functionality) of a cellular component. Static regulation maintains expression and/or activity at some given level. Upregulation refers to a relative increase in expression and/or activity. Accordingly downregulation refers to a relative decrease in expression and/or activity. In the present invention, regulation is preferably downregulation of a cellular component. As used herein,

downregulation is synonymous with inhibition of a given cellular component's activity.

Therapeutics, pharmaceutically active agents or inhibitors, respectively, may be administered to cells from an individual *in vitro*, or may involve *in vivo* administration to the individual. The term "individual" preferably refers to mammals and most preferably to humans. Routes of administration of pharmaceutical preparations to an individual may include oral and parenteral, including dermal, intradermal, intragastral, intracutan, intravasal, intravenous, intramuscular, intraperitoneal, intranasal, intravaginal, intrabuccal, percutan, rectal, subcutaneous, sublingual, topical or transdermal application, but are not limited the these ways of administration. For instance, the preferred preparations are in administratable form which is suitable for oral application. These administratable forms, for example, include pills, tablets, film tablets, coated tablets, capsules, powders and deposits. Administration to an individual may be in a single dose or in repeated administrations, and may be in any of a variety of physiologically acceptable salt forms, and/or with an acceptable pharmaceutical carrier, binder, lubricant, excipient, diluent and/or adjuvant. Pharmaceutically acceptable salt forms and standard pharmaceutical formulation techniques are well known to persons skilled in the art.

As used herein, a "pharmaceutical effective amount" of a human cellular protein kinase, metalloprotease or phosphatase inhibitor is an amount effective to achieve the desired physiological result, either in cells treated *in vitro* or in a subject treated *in vivo*. Specifically, a pharmaceutically effective amount is an amount sufficient to inhibit, for some period of time, one or more of the clinically defined pathological processes associated with the viral infection. The effective amount may vary depending on the specific human cellular protein kinase, metalloprotease or phosphatase inhibitor selected, and is also dependent on a variety of factors and conditions related to the subject to be treated and the severity of the infection. For example, if the inhibitor is to be administered *in vivo*, factors such as the age, weight and health of the patient as well as dose response curves and toxicity data obtained in pre-clinical animal work would be among

those considered. If the inhibitor is to be contacted with the cells *in vitro*, one would also design a variety of pre-clinical *in vitro* studies to assess such parameters as uptake, half-life, dose, toxicity, etc. The determination of a pharmaceutically effective amount for a given agent is well within the ability of those skilled in the art.

It is also apparent to a person skilled in the art that detection includes any method known in the art useful to indicate the presence, absence, or amount of a detection target. Such methods may include, but are not limited to, any molecular or cellular techniques, used singularly or in combination, including, but not limited to: hybridization and/or binding techniques, including blotting techniques and immunoassays; labeling techniques (chemiluminescent, colorimetric, fluorescent, radioisotopic); spectroscopic techniques; separations technology, including precipitations, electrophoresis, chromatography, centrifugation, ultrafiltration, cell sorting; and enzymatic manipulations (e.g., digestion).

The present disclosure teaches for the first time the up- or downregulation of a group of human cellular protein kinases, metalloproteases and a phosphatase specifically involved in the viral infection of Hepatitis C virus. Thus, the present invention is also directed to a method useful for detecting novel compounds useful for prophylaxis and/or treatment of HCV infections.

Methods of the present invention identify compounds useful for prophylaxis and/or treatment of HCV infections by screening a test compound, or a library of test compounds, for its ability to inhibit any one or more of the group of human cellular protein kinases, metalloproteases or phosphatases identified herein as characteristically up- or downregulated during HCV growth and RNA replication inside a cell. A variety of assay protocols and detection techniques are well known in the art and easily adapted for this purpose by a skilled practitioner. Such methods include, but are not limited to, high throughput assays (e.g., microarray technology, phage display technology), and *in vitro* and *in vivo* cellular and tissue assays.

In a related aspect, the present invention provides, in view of the discovery of human cellular protein kinases, metalloproteases and phosphatases specifically involved in the HCV infection process, an assay component specially useful for detecting HCV in an individual or in cells. Preferably the assay component comprises oligonucleotides immobilized on a solid support capable of detecting activity of one or more of the human cellular protein kinases, metalloproteases or phosphatase comprising:

the kinases	beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1 (NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265);
the metalloproteases	ADAM22 (NM_016351), ADAM17 (U92649) and
the phosphatase	MKP-L (NM_007026).

Preferably the solid support would contain oligonucleotides of sufficient quality and quantity to detect all of the above-mentioned human cellular proteins (e.g., a nucleic acid microarray).

Similarly, it is an object of the present invention to provide an assay component specially useful for screening compounds useful for the prophylaxis and/or treatment of HCV infections. One preferred assay component comprises oligonucleotides that encode one or more human cellular protein kinases: beta-adrenergic receptor kinase (X61157), Mitogen activated protein kinase activated protein kinase (AF032437), Insulin-stimulated protein kinase 1 (U08316), TRK E (X74979), PKC-mu (X75756), PKC-theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (L31951), Human p21-activated protein kinase, and PAK2 (U24153); or metalloproteases: ADAM22 (AF155382) and ADAM17

(XM_002270); or the Phosphatase MKP-L, (NM_007026) immobilized on a solid support.

In another embodiment, the assay component comprises peptide fragments of one or more of the above-identified human cellular proteins immobilized on a solid support. Once again the most preferred solid support embodiment would contain polymers of sufficient quality and quantity to detect all of the above-mentioned human cellular protein kinases, metalloproteases and the phosphatase (e.g., a nucleic acid or a peptide microarray). A variety of supports and constructions of the same for the methods disclosed herein are well known in the art and easily adapted for this purpose by a skilled practitioner (cf., for example: Marschall, 1999 "Do-it-yourself gene watching" Science 286, 444-447; Service 2000 "Protein arrays step out of DNA's shadow" Science 289, 1673).

It is preferred that mRNA is measured as an indication of expression. Methods for assaying for mRNA include, but are not limited to, Nothern blots, slot blots, dot blots, and hybridization to an ordered array of oligonucleotides. Nucleic acid probes useful for assay of a sample are preferably of sufficient length to specifically hybridize only to appropriate, complementary transcripts. Typically the oligonucleotide probes will be at least 10 to 25 nucleotides in length. In some cases longer probes of at least 30, 40, or 50 up to 2500 nucleotides will be desirable.

The polypeptide product of gene expression may be assayed to determine the amount of expression as well. Methods for assaying for a protein include, but are not limited to, western blot, immuno-precipitation, radioimmuno assay, and peptide immobilization in an ordered array. It is understood, however, that any method for specifically and quantitatively measuring a specific protein or mRNA product can be used.

A variety of supports upon which nucleic acids or peptides can be immobilized are known in the art, for example filters, or polyvinyl chloride dishes. Any solid surface to which oligonucleotides or peptides can be bound, either directly or indirectly,

either covalently or non-covalently, can be used. A preferred solid support is a microarray membrane filter or a "biochip". These contain particular polymer probes in predetermined locations on the array. Each predetermined location may contain more than one molecule of the probe, but each molecule within the predetermined location has an identical sequence.

The present invention incorporates by reference in their entirety techniques well known in the field of molecular biology. These techniques include, but are not limited to, techniques described in the following publications:

Ausubel, F.M. et al. eds., "Short Protocols In Molecular Biology" 4th Ed. 1999, John Wiley & Sons, NY (ISBN 0-471-32938-X);

Old, R.W. & S.B. Primrose "Principles of Gene Manipulation: An Introduction To Genetic Engineering" 3rd Ed. 1985, Blackwell Scientific Publications, Boston. Studies in Microbiology: V.2, 409 pp. (ISBN 0-632-01318-4);

Miller, J.H. & M.P. Calos eds., "Gene Transfer Vectors For Mammalian Cells" 1987, Cold Spring Harbor Laboratory Press, NY. 169 pp. (ISBN 0-87969-198-0);

Mayer, R.J. & J.H. Walker eds. "Immunochemical Methods In Cell and Molecular Biology" 1987, Academic Press, London. 325 pp. (ISBN 0-12480-855-7);

Sambrook, J. et al. eds., "Molecular Cloning: A Laboratory Manual" 2nd Ed. 1989, Cold Spring Harbor Laboratory Press, NY. Vols. 1-3. (ISBN 0-87969-309-6);

Winnacker, E.L. "From Genes To Clones: Introduction To Gene Technology" 1987 VCH Publishers, NY. (translated by Horst Ibelgaufts) 634 pp. (ISBN 0-89573-614-4).

The present invention further incorporates by reference in their entirety techniques well known in the field of microarray construction and analysis. These techniques include, but are not limited to, techniques described in the following patents and patent applications describing array of biopolymeric compounds and methods for their fabrication:

U.S. Pat. Nos. 5,242,974; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,436,327; 5,445,934; 5,472,672; 5,527,681; 5,529,756; 5,545,531; 5,554,501; 5,556,752; 5,561,071; 5,559,895; 5,624,711;

5,639,603; 5,658,734; 5,807,522; 6,087,102; WO 93/17126; WO 95/11995; WO 95/35505; EP 742 287; and EP 799 897.

Techniques also include, but are not limited to, techniques described in the following patents and patent application describing methods of using arrays in various applications:

U.S. Pat. Nos. 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,994,076; 6,033,860; 6,040,138; 6,040,140; WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280

It is readily apparent to those skilled in the art that other suitable modifications and adaptations of the compositions and methods of the invention described herein are evident and may be made without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

Examples

Materials and Methods

1. Generation of cDNA-arrays on membranes

In order to manufacture cDNAs-arrays on membranes, the following strategy was pursued: cDNAs encoding parts of or full length proteins of interest – in the following referred to as "target cDNAs" – were cloned into the plasmid Bluescript II KS$^+$ (Stratagene, USA). Large scale purifications of these plasmids were performed according to standard techniques and 200 µl aliquots (1 µg/µl plasmid concentration) were transferred into appropriate 96 well plates. Plates were closed with sealing tape and chilled on ice for 5 minutes after incubation for 10 minutes at 95°C. 10 µl of 0.6N NaOH were added and the mix was stored for 20

minutes at room temperature before addition of 10 µl 2.5M Tris-HCl pH 7.1 and 20 µl 40x SSC. Target cDNAs were spotted onto Nylon or Nitrocellulose membranes using a BioGrid (BioRobotics, UK) equipped with a 0.7 mm pintool. In this way, between 200 ng and 350 ng of plasmids encoding target cDNAs were transferred onto the membranes and crosslinked to the membranes by ultraviolet light ($1.2x10^5$ µJ/cm^2). The arrays were stored for use in subsequent experiments at at 4°C.

2. Cellular HCV RNA replication system

Huh-pcDNA3, Huh-9-13, Huh-5-15 and Huh-11-7 cells were grown in DMEM supplemented with 10% FCS, 2 mM Glutamine, Penicillin (100 IU/ml) / Streptomycin (100 µg/ml) and 1x nonessential amino acids in the presence of 1 mg/ml G418. Cells were routinely passaged three times a week at a dilution of 1:3 or 1:2.

3. Lysis of cells, and isolation of total RNA

Huh-pcDNA3, Huh-9-13, Huh-5-15 and Huh-11-7 cells were seeded at 5×10^5 cells per 10 cm plate in medium without G148. The medium was changed 3 days after plating and cells were harvested 5 days after plating by lyzing the cells directly on the plate with 4 ml of Tri reagent (Molecular Research Center, Inc., USA). The lysates were stored at room temperature for 5 minutes and then centrifuged at 12000xg for 15 minutes at 4°C. The supernatant was mixed with 0,1 ml of 1-Bromo-3-chloropropane per 1 ml of Tri reagent and vigorously shaken. The suspension was stored for 5 minutes at room temperature and then centrifuged at 12000xg for 15 minutes at 4°C. The colorless upper phase was transferred into new tubes, mixed with 5 µl of polyacryl-carrier (Molecular Research Center Inc., USA) and with 0.5 ml of isopropanol per 1 ml of Tri reagent and vigorously shaken. The samples were stored at room temperature for 5 minutes and then centrifuged at 12000xg for 8 minutes at 4°C. The supernatant was removed and the RNA pellet washed twice with 1 ml of 75% Ethanol. The pellet was dried and resuspended in 25 µl of RNase-free buffer per initial 1 ml lysate.

4. Preparation of radioactively labelled cDNA probes from RNA

In order to obtain a radioactively labeled cDNA probe, RNA was transcribed into a cDNA-probe in the presence of radioactively labeled dATP. 12 µl bidestilled DEPC treated H_2O containing 1 µg of primer TXN (5'-TTT TTT TTT TTT TTT TXN-3' with T → dTTP; N → dATP, dCTP, dGTP or dTTP; X → dATP, dCTP or dGTP) and total RNA (6 µg) were shaken between 5 and 15' at 60°C and then incubated at 4°C for 2 to 10 minutes. After centrifugation (30 seconds, 10000xg) 7 µl of a mix consisting of 100 µCi dATP-P^{33} (Amersham, UK) which were dried under vacuum previously and resuspended in 4 µl first strand buffer (Life Technologies, USA), 2 µl 0.1M DTT and 1 µl labeling solution (4mM dCTP, dGTP, dTTP each and 80 µM dATP final concentration) were added. Following the addition of 1 µl Superscript II reverse transcriptase (Life Technologies, USA) the reaction was incubated for 10 minutes at room temperature and then for 60 minutes at 38°C. Subsequently, the reaction was vigorously shaken for 30 minutes at 68°C after adding 5 µl 0.5M EDTA and 25 µl 0.6M NaOH.

Unincorporated nucleotides were removed from the labeling reaction using ProbeQuant G-50 columns (Amersham, UK). The column was vigorously shaken and centrifuged for 1 minute at 735xg in an appropriate reaction tube after bottom closure and lid were removed. The column was placed into a new reaction tube, the probe was applied onto the center of the column material and the column was centrifuged for 2 minutes at 735xg. The flow-trough was transferred into new reaction tubes and filled up to a volume of 100 µl with 10mM Tris, pH 7.4, 1 mM EDTA. The probe was precipitated by centrifugation for 15 minutes at 12000xg after 4 µl of 5M NaCl, 1 µl polyacryl-carrier (Molecular Research Center Inc., USA) and 250µl Ethanol were added. The supernatant was discarded and the pellet dried before starting with the hybridisation.

5. Hybridisation of radioactively labeled cDNA-probes to cDNA-arrays

The pellet was resuspended in 10 µl C_0T DNA (1 µg/µl, Roche Diagnostics, Germany), 10 µl yeast tRNA (1 µg/µl Sigma, USA) and 10 µl polyA (1 µg/µl, Roche

Diagnostics, Germany). Herring sperm DNA was added to a final concentration of 100 µg/ml and the volume was filled up to 100 µl with 5 µl 10% SDS (Sodiumdodecylsulfat), 25 µl 20x SSPE and bidestilled H_2O. The mix was put on 95°C for 5 minutes, centrifuged for 30 seconds at 10000xg and vigorously shaken for 60 minutes at 65°C. A 1 µl aliquot of the probe was used to measure the incorporation of radioactive dATP with a scintillation counter. Probes with at least a total of $20x10^6$ cpm were used. The arrays were prehybridised for at least 3 hours at 65°C in hybridisation solution in a roller bottle oven. After prehybridisation the radioactively labeled probe was added into the hybridisation solution and hybridisation was continued for 20 hours. The probe was discarded and replaced with wash solution A (2xSSC). The arrays were washed twice in wash solution A at room temperature in the roller oven. Afterwards, wash solution A was replaced by wash solution B (2x SSC, 0.5% SDS) preheated to 65°C and arrays were washed twice for 30 minutes at 65°C. Then, wash solution B was replaced by wash solution C (0.5x SSC, 0.5% SDS) preheated to 65°C and arrays were washed twice for 30 minutes at 65°C. The moist arrays were wrapped in airtight bags and exposed for 8 to 72 hours on erased phosphoimager screens (Fujifilm, Japan).

6. Analysis of cDNA-arrays

The exposed phosphoimager screens were scanned with a resolution of 100 µ and 16bits per pixel using a BAS-1800 (Fujifilm, Japan). Files were imported into the computer program ArrayVision (Imaging Research, Canada). Using the program's features, the hybridisation signals of each target cDNA were converted into numbers. The strength of the hybridisation signals reflected the quantity of RNA molecules present in the probe. Differentially expressed genes were selected according to the ratio of their signal strength after normalization to the overall intensity of the arrays.

7. Analysis of expression levels by Northern blot experiment

Huh-pcDNA3, Huh-9-13, Huh-5-15 and Huh-11-7 cells were seeded at 5×10^5 cells per 10 cm plate in medium without G148. Cells were harvested after 3 days

by lyzing the cells directly on the plate with 4 ml of Tri reagent (Molecular Research Centre, Inc., USA). The lysates were stored at room temperature for 5 minutes and then centrifuged at 12000xg for 15 minutes at 4°C. The supernatant was mixed with 0,1 ml of 1-bromo-3-chloropropane per 1 ml of Tri reagent and vigorously shaken. The suspension was stored for 5 minutes at room temperature and then centrifuged at 12000xg for 15 minutes at 4°C. The colourless upper phase was transferred into new tubes, mixed with 5 µl of poly-acryl-carrier (Molecular Research Centre, Inc., USA) and with 0.5 ml of isopropanol per 1 ml of Tri reagent and vigorously shaken. The samples were stored at room temperature for 5 minutes and then centrifuged at 12000xg for 8 minutes at 4°C. The supernatant was removed and the RNA pellet washed twice with 1 ml of 75% Ethanol. The pellet was dried and resuspended in 25 µl of RNase-free water per inital 1 ml lysate. 8 µg of total RNA per sample was loaded onto formaldehyde-containing agarose gels (Sambrook et al. Cloning manual, CSHL press, 1989) and transferred to HYbond NX membranes (Amersham) overnight in 20x SSC (3M NaCl, 300mM $C_6H_5Na_3O_7$ x 2 H_2O, pH 7.0) by capillary transfer. RNA was immobilized to the filter using UV-crosslinking (120mJ/cm^2 for 25 seconds). Filters were hybridized to oligonucleotide probes or random-primed probes specific for the genes in question. Quantitation of signals was performed with a Fuji phosphoimager.

8. Verification of de-regulated genes by quantitative Real-Time PCR

Quantitative RT-PCR was used to verify hits resulting from DNA macroarray experiments by exploiting the 5'-exonulease of Taq DNA polymerases to cleave the 5' fluorescent label of an oligonucleotide. Total RNA was extracted from cell lines (Qiagen RNeasy Mini Kit, QIAGEN, Hilden) and was reverse transcribed with Superscript II (Invitrogen, Karlsruhe) according to the manufacturer's protocol with 5 µg of RNA as a template and oligodT primers. Subsequently, the cDNA was analysed on a ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Darmstadt) with the 5'exonuclease assay by using the TaqMan Universal PCR Master Mix (#4324018, Applied Biosystems, Darmstadt) and non-extendible oligonucleotides. Gene-specific TaqMan probes were labelled with the reporter dye FAM™ at the 5'-end and the quencher dye TAMRA™ at the 3' end of

the probe. GAPDH and 18SrRNA were used as reference genes with TaqMan probes that were labelled with VIC™ and TAMRA™ accordingly.
Experimental conditions were 2 minutes 50°C, 10 minutes 95°C, followed by 40 cycles with 15 seconds at 95°C and 1 minute at 60°C. Primer Express software was used to design primers with a melting temperature of 58 - 60°C amplifying an amplicon of a maximum length of 150 bp.

9. Analysis of expression levels by Western blot experiments

Huh-pcDNA3, Huh-9-13, Huh-5-15 and Huh-11-7 cells were seeded at 5 x 10^5 cells per 10 cm plate in medium without G148. Cells were harvested after 3 days by the addition of 500 µl of 1x SDS sample buffer (62.5 mM Tris-HCl pH 6.8, 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% bromphenol blue) or RIPA lysis buffer. Lysates were separated on SDS-poly acrylamide gels and proteins transfered to nitrocellulose. Western blotting was performed with the appropriate antibodies according to the manufacturers instructions.

Claims

1. A method for detecting compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infections, the method comprising the following steps:

   a) contacting a test compound with at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), ADAM17 (U92649); and

   b) determining the activity of said human cellular protein kinase, metalloprotease or phosphatase.

2. A method for detecting Hepatitis C virus infections in an individual, the method comprising the following steps:

   a) providing a sample from said individual; and

   b) determining the activity in said sample of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4

(U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

3. A method for detecting Hepatitis C virus infections in cells, the method comprising the following steps:
   a) providing said cells; and
   b) determining the activity in said cells of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

4. An antibody that binds to a human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

5. The antibody of claim 4, wherein said antibody is a monoclonal or polyclonal antibody.

6. A method for treating Hepatitis C virus infection in an individual, the method comprising the step of administering a pharmaceutically effective amount of an

agent which inhibits at least partially the activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

7. A method for regulating the production of Hepatitis C virus in cells, the method comprising the step of administering a pharmaceutically effective amount of an agent to said cells wherein said agent inhibits at least partially the activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

8. The method according to claim 6 or 7, wherein the agent is an antibody which binds to at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein

kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

9. The method according to claim 8, wherein said antibody is a monoclonal or polyclonal antibody.

10. A solid support useful for detecting Hepatitis C virus infections in an individual, the solid support comprising an immobilized oligonucleotide, wherein said oligonucleotide is capable of detecting activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

11. A solid support useful for detecting Hepatitis C virus infections in cells, the solid support comprising an immobilized oligonucleotide, wherein said oligonucleotide is capable of detecting activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

12. A solid support useful for screening compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infections in an individual, the solid support comprising at least one immobilized oligonucleotide, wherein said oligonucleotide encodes one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

13. A solid support useful for screening compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infections in an individual, the solid support comprising at least one immobilized human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

14. A composition useful for the prophylaxis and/or treatment of an individual afflicted with Hepatitis C virus, the composition comprising at least one agent capable of inhibiting activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM_013994), Protein

Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM_007026), ADAM22 (NM_016351), and ADAM17 (U92649).

10. Nucleic acid and amino acid sequences of human cellular proteins:

DEFINITION Homo sapiens adrenergic, beta, receptor kinase 1 (ADRBK1), mRNA.
ACCESSION NM_001619
EC number = "2.7.1.126

DEFINITION Homo sapiens mitogen activated protein kinase activated protein
kinase gene, complete cds.
ACCESSION AF032437
VERSION AF032437.1 GI:3133290
FEATURES Location/Qualifiers
source 1..1968
/organism="Homo sapiens"
/db_xref="taxon:9606"
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BASE COUNT 571 a 445 c 485 g 467 t
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DEFINITION  Human insulin-stimulated protein kinase 1 (ISPK-1) mRNA,
complete
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BASE COUNT 704 a 417 c 510 g 629 t

ORIGIN

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DEFINITION Homo sapiens discoidin domain receptor family, member 1 (DDR1),
transcript variant 3, mRNA.
ACCESSION NM_013994
VERSION NM_013994.1 GI:7669484
EC number = "2.7.1.112"

DEFINITION H.sapiens mRNA for protein kinase C mu.
ACCESSION X75756
VERSION X75756.1 GI:438372
FEATURES Location/Qualifiers

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BASE COUNT     1015 a    897 c    865 g    965 t

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DEFINITION  Human protein kinase C-theta (PRKCT) mRNA, complete cds.
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BASE COUNT 764 a 657 c 686 g 647 t

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2701 ctgtggatgg gtaaatgctg ggggccagcc ctggataggt ttttatggga attc
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DEFINITION  Homo sapiens mRNA for AMP-activated protein kinase beta 2
subunit.
ACCESSION   AJ224538
VERSION     AJ224538.1  GI:2916801
FEATURES             Location/Qualifiers
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                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
       gene           1..819
                     /gene="AMPK beta 2"
       CDS            1..819
                     /gene="AMPK beta 2"
                     /function="regulatory subunit"
                     /codon_start=1
                     /evidence=experimental
                     /product="AMP-activated protein kinase beta 2
subunit"
                     /protein_id="CAA12030.1"
                     /db_xref="GI:2916802"
                     /db_xref="SWISS-PROT:O43741"

/translation="MGNTTSDRVSGERHGAKAARSEGAGGHAPGKEHKIMVGSTDDPS

VFSLPDSKLPGDKEFVSWQQDLEDSVKPTQQARPTVIRWSEGGKEVFISGSFNNWSTK

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EVFDALKLDSMESSETSCRDLSSSPPGPYGQEMYAFRSEERFKSPPILPPHLLQVILN

KDTNISCDPALLPEPNHVMLNHLYALSIKDSVMVLSATHRYKKKYVTTLLYKPI"
BASE COUNT      208 a    215 c    197 g    199 t

ORIGIN
     1 atgggaaaca ccaccagcga ccgggtgtcc ggggagcgcc acggcgccaa ggctgcacgc
    61 tccgagggcg caggcggcca tgccccgggg aaggagcaca agatcatggt ggggagtacg
   121 gacgacccca gcgtgttcag cctccctgac tccaagctcc ctggggacaa agagtttgta
   181 tcatggcagc aggatttgga ggactccgta aagcccacac agcaggcccg gcccactgtt
   241 atccgctggt ctgaaggagg caaggaggtc ttcatctctg ggtctttcaa caattggagc
   301 accaagattc cactgattaa gagccataat gactttgttg ccatcctgga cctccctgag
   361 ggagagcacc aatacaagtt ctttgtggat ggacagtggg ttcatgatcc atcagagcct
   421 gtggttacca gtcagcttgg cacaattaac aatttgatcc atgtcaagaa atctgatttt
   481 gaggtgttcg atgctttaaa gttagattct atggaaagtt ctgagacatc ttgtagagac
   541 ctttccagct caccccccagg gccttatggt caagaaatgt atgcgtttcg atctgaggaa
   601 agattcaaat ccccacccat ccttcctcct catctacttc aagttattct taacaaagac
   661 actaatattt cttgtgaccc agccttactc cctgagccca accatgttat gctgaaccat
   721 ctctatgcat tgtccattaa ggacagtgtg atggtcctta gcgcaaccca tcgctacaag
   781 aagaagtatg ttactactct gctatacaag cccatttga
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DEFINITION Human protein kinase (JNK2) mRNA, complete cds.
ACCESSION U09759
VERSION U09759.1 GI:607785
FEATURES Location/Qualifiers
source 1..1873
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/db_xref="taxon:9606"
gene 152..1426
/gene="JNK2"
CDS 152..1426
/gene="JNK2"
/codon_start=1
/product="protein kinase"
/protein_id="AAA74740.1"
/db_xref="GI:607786"

/translation="MSDSKCDSQFYSVQVADSTFTVLKRYQQLKPIGSGAQGIVCAAF
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DVYLVMELMDANLCQVIHMELDHERMSYLLYQMLCGIKHLHSAGIIHRDLKPSNIVVK
SDCTLKILDFGLARTACTNFMMTPYVVTRYYRAPEVILGMGYKENVDIWSVGCIMGEL
VKGCVIFQGTDHIDQWNKVIEQLGTPSAEFMKKLQPTVRNYVENRPKYPGIKFEELFP
DWIFPSESERDKIKTSQARDLLSKMLVIDPDKRISVDEALRHPYITVWYDPAEAEAPP
PQIYDAQLEEREHAIEEWKELIYKEVMDWEERSKNGVVKDQPPDAAVSSNATPSQSSS
INDISSMSTEQTLASDTDSSLDASTGPLEGCR"
BASE COUNT 562 a 388 c 427 g 496 t
ORIGIN

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  61 gtccacgccg cggtaggtga agttgcggaa ggtccggcga gggatctgaa acttgccccc
 121 taccttcgg gatattgcag gacgctgcat catgagcgac agtaaatgtg acagtcagtt
 181 ttatagtgtc caagtggcag actcaaccct cactgtccta aaacgttacc agcagctgaa
 241 accaattggc tctggggccc aagggattgt ttgtgctgca tttgatacag ttcttgggat
 301 aaatgttgca gtcaagaaac taagccgtcc ttttcagaac caaactcatg caaagagagc
 361 ttatcgtgaa cttgtcctct taaaatgtgt caatcataaa aatataatta gtttgttaaa
 421 tgtgtttaca ccacaaaaaa ctctagaaga atttcaagat gtgtatttgg ttatggaatt
 481 aatggatgct aacttatgtc aggttattca catggagctg gatcatgaaa gaatgtccta
 541 ccttctttac cagatgcttt gtggtattaa acatctgcat tcagctggta taattcatag
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 721 ctaccgggcg cccgaagtca tcctgggtat gggctacaaa gagaacgttg atatctggtc
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1081 ctctgtagac gaagctctgc gtcacccata catcactgtt tggtatgacc ccgccgaagc
1141 agaagcccca ccacctcaaa tttatgatgc ccagttggaa gaaagagaac atgcaattga
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1741 attgtgcatg tgaccacaaa tgcttgcttg gacttgccca tctagcactt tggaaatcag
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DEFINITION Human p21-activated protein kinase (Pak2) gene, complete cds.

ACCESSION U24153
VERSION U24153.1 GI:780807
FEATURES Location/Qualifiers
source 1..2019
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/db_xref="taxon:9606"
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/clone_lib="lambdaYESR human lymphocyte cDNA library;
Elledge and Spottswood, EMBO 10:2653(1991)"
gene 40..1617
/gene="Pak2"
CDS 40..1617
/gene="Pak2"
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/product="p21-activated protein kinase"
/protein_id="AAA65442.1"
/db_xref="GI:780808"

/translation="MSDNGELEDKPPAPPVRMSSTIFSTGGKDPLSANHSLKPLPSVP
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GTEAPAVVTEEEDDDEETAPPVIAPRPDHTKSIYTRSVIDPVPAPVGDSHVDGAAKSL
DKQKKKPKMTDEEIMEKLRTIVSIGDPKKKYTRYEKIGQGASGTVFTATDVALGQEVA
IKQINLQKQPKKELIINEILVMKELKNPNIVNFLDSYLVGDELFVVMEYLAGGSLTDV
VTETACMDEAQIAAVCRECLQALEFLHANQVIHRDIKSDNVLLGMEGSVKLTDFGFCA
QITPEQSKRSTMVGTPYWMAPEVVTRKAYGPKVDIWSLGIMAIEMVEGEPPYLNENPL
RALYLIATNGTPELQNPEKLSPIFRDFLNRCLEMDVEKRGSAKELLQHPFLKLAKPLS
SLTPLIMAAKEAMKSNR"
BASE COUNT 615 a 413 c 481 g 510 t

ORIGIN

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  61 gaagataagc ctccagcacc tcctgtgcga atgagcagca ccatctttag cactggaggc
 121 aaagaccctt tgtcagccaa tcacagtttg aaacctttgc cctctgttcc agaagagaaa
 181 aagcccaggc ataaaatcat ctccatattc tcaggcacag agaaaggaag taaaaagaaa
 241 gaaaaggaac ggccagaaat ttctcctcca tctgattttg agcacaccat ccatgttggc
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 661 gattcacatg ttgatggtgc tgccaagtct ttagacaaac agaaaaagaa gcctaagatg
 721 acagatgaag agattatgga gaaattaaga actatcgtga gcataggtga ccctaagaaa
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DEFINITION Human cyclin-dependent kinase 4 (CDK4) gene, complete cds.

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ACCESSION   U37022
VERSION     U37022.1  GI:1353415
FEATURES             Location/Qualifiers
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       exon           85..291
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       exon           729..965
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                     3135..3270,3836..3928)
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                     /db_xref="GI:1353416"

/translation="MATSRYEPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGG

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LARIYSYQMALTPVVVTLWYRAPEVLLQSTYATPVDMWSVGCIFAEMFRRKPLFCGNS
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BASE COUNT 945 a 1050 c 1101 g 1124 t 13 others
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61 cccagaacgt ccggcgttcg ccccgccctc ccagtttccg cgcgcctctt tggcagctgg
121 tcacatggtg agggtggggg tgaggggccc tctctagctt gcggcctgtg tctatggtcg
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421 gaaagtataa ttttagggac tgaggtgtag gatcttcgat gcaaggcatg tgtcatgtgt
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DEFINITION Human MEK5 mRNA, complete cds.
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VERSION U25265.1 GI:1255719
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BASE COUNT 518 a 532 c 512 g 521 t
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DEFINITION  Homo sapiens a disintegrin and metalloproteinase
domain 22
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VERSION     NM_016351.2  GI:11497041
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BASE COUNT 1000 a 641 c 779 g 891 t 3 others

ORIGIN

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            variant 1, mRNA.
ACCESSION   NM_003183
VERSION     NM_003183.3  GI:11497003
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BASE COUNT 1025 a 685 c 845 g 968 t

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Accession number: NM_007026

Definition: DUSP14, MKP-L

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AAATGGCTGACTTCTGGTTCTCCCTCAAGTGTTTTTTACACTGGGTGTTCAAATTTATTTTAAGAGATAG
GGAGGGAGGGGACATAAAGGGAATGCATACATTGCTAGTCACATTTTTAAAATTAACATTTTGGAATAGT
GTTTATGGAAATCTTTAGCTTTTAATCATTTTTACCAATTTGAACAGTTTAATAAACTGGTTCTGCTCTC
TTCTGAATCTCATGCCTTTGGCACCTTGGTAGGTGCAGGAGGAGCTCAGTGCAAAAATCACTTTGGGGCC
TCATTAACCCTTTAGAGACAAGCTTTGCCCCAGGCTGCGGACCAGACAGATGCTTAGGGAAGGTTGATAC
CAGCTTCAGTCTCTACTGGATTAGCCCTACTCTTTCCTTTCCCCTCCATTATTTAGTGACTCTGTAAGTA
AGTTAAATACACCCTTATTATTTAGCTGTTAAGTAACTATAATGAAATCTGCTGCAAAATCTCTCTTGGA
ATCCATGTGCCCAGGATTATATTAGCATTATTTTTAATAAATCTATATGCTTAAAAAAAAAAAAAAAAAA
A

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WO 2003/054228 A3

(54) Title: HUMAN CELLULAR ENZYMES AS TARGETS AGAINST HEPATITIS C VIRUS INFECTIONS

(57) Abstract: The present invention relates to human cellular protein kinases, metalloproteases and one phosphatase: beta-adrenergic receptor kinase 1 (NM 001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM 013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM 007026), ADAM22 (NM 016351), and ADAM17 (U92649) as potential targets for medical intervention against Hepatitis C virus (HCV) infections. The present invention relates also to a method for the detection of compounds useful for prophylaxis and/or treatment of Hepatitis C virus infections, a method for detecting Hepatitis C virus infections in an individual or in cells. Mono- or polyclonal antibodies are disclosed effective for the treatment of HCV infections together with methods for treating Hepatitis C virus infections or for the regulation of Hepatitis C virus production and/or replication wherein said antibodies may be used. Finally the present invention relates to a solid support useful for detecting Hepatitis C virus infections or for screening compounds useful for prophylaxis and/or treatment of HCV infections.

INTERNATIONAL SEARCH REPORT

itional Application No
PCT/EP 02/14578

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/42 C12Q1/48 C12Q1/37 C12Q1/68 C07K16/00
A61K39/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, EMBASE, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRANCH ANDREA D ET AL: "The coming impact of gene expression profiling on the diagnosis and treatment of HCV-associated liver disease." ANTIVIRAL RESEARCH, vol. 52, no. 2, November 2001 (2001-11), pages 173-179, XP002249604 ISSN: 0166-3542 the whole document ---	1-14
X	BIGGER CATHERINE B ET AL: "DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection." JOURNAL OF VIROLOGY, vol. 75, no. 15, August 2001 (2001-08), pages 7059-7066, XP002249605 ISSN: 0022-538X the whole document ---	1-14
	-/--	

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 July 2003	21/08/2003
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer Niemann, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

itional Application No
PCT/EP 02/14578

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YAMASHITA TARO ET AL: "Serial analysis of gene expression in chronic hepatitis C and hepatocellular carcinoma." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 282, no. 2, 30 March 2001 (2001-03-30), pages 647-654, XP002249606 ISSN: 0006-291X the whole document	1-14
X	HUANG YING ET AL: "A human hepatoma cell line expressing hepatitis C virus nonstructural proteins tightly regulated by tetracycline." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 281, no. 3, 2 March 2001 (2001-03-02), pages 732-740, XP002249607 ISSN: 0006-291X the whole document	1-14

INTERNATIONAL SEARCH REPORT

...ternational application No.
PCT/EP 02/14578

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 2, 3. are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Although claims 6-9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition

Continuation of Box I.2

Present claims 6-7, 14 relate to an agent defined by reference to a desirable characteristic or property, namely inhibiting at least aprtially the activity of at least one of the enzymes cited in claim 1.

The claims cover all agents having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such agent. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the agent by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to antibody which binds to at least one of the enzymes cited in claim 1 (see claim 8).

Present claims 10-11 relate to an oligonucleotide defined by reference to a desirable characteristic or property, namely being capable ofdetecting activity of at least one of the enzymes cited in claim 1.

The claims cover all oligonucleotides having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such oligonucleotide. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the oligonucleotide by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to oligonucleotides encoding one of the enzymes cited in claim 1 (see claim 12).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1 : 1-14 (all partially)

method for detecting compounds useful for the prophylaxis and/or treatment of Hepatits C virus infection, the method comprising contacting a test compound with at least beta-adrenergic receptor kinase 1
method for detecting Hepatitis C virus infection

Inventions 2-14 : 1-14 (all partially)

method for detecting compounds useful for the prophylaxis and/or treatment of Hepatits C virus infection, the method comprising contacting a test compound with at least one of the enzymes mentioned in claim 1
method for detecting Hepatitis C virus infection

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